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*University of Arkansas, Fayetteville*

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Effect of Potassium Deficiency on Uptake and Partitioning in the Cotton (*Gossypium hirsutum*  
L.) Plant and Detection by a Crop Reflectance Sensor

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science in Crop, Soil, and Environmental Sciences

by

Taylor Dayne Coomer  
University of Arkansas  
Bachelor of Science in Crop, Soil, and Environmental Sciences, 2013

May 2016  
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

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## Abstract

For cotton (*Gossypium hirsutum* L.) to grow and develop normally, plants need to uptake the necessary amount of nutrients and use those nutrients in a beneficial fashion. It is recognized that cotton needs a certain tissue concentration of ions to achieve and maintain growth rates (Siddiqi et al., 1987). One of the most essential and abundant nutrients in cotton is potassium (K), second only by mass to nitrogen (N) (Marschner, 1995; Oosterhuis et al., 2013). Potassium exists in the soil in four separate pools and moves through soil to roots mainly through diffusion (Rengel & Damon, 2008; Samal et al., 2010; Ogaard et al., 2001). Potassium plays a vital role in plant growth and metabolism.

The objectives of this study were to determine the Michaelis-Menten parameters for the high-affinity transport system (HATS) and low-affinity transport system (LATS) uptake mechanisms of cotton, observe how K is partitioned throughout the cotton plant over a growing season with differing K fertilization rates, and to determine if cultivars differed in values from currently available indices formulated for N-status detection from active sensors. It also set out to determine if these N-sensitive indices were sensitive to leaf K concentration and available  $K_2O$  in the soil, and to evaluate the role these indices play in predicting yield. It was hypothesized that a high K hydroponic environment would lead to more K uptake by cotton roots, which would lead to an increase in  $V_{MAX}$  and  $K_M$ . It was also hypothesized that with increased K fertilization, there would be greater K uptake and larger shift to reproductive components due to the plant having more than enough K in all other parts enabling it to send more to the reproductive components, and that greater K rates would lead to higher yields across all cultivars. It was believed that normalized difference vegetation index (NDVI) would more accurately predict leaf K, available  $K_2O$ , and yield than normalized difference red edge (NDRE),

that NDVI and NDRE would more accurately determine the K parameters chosen than canopy chlorophyll content index (CCCI), due to the strong influence of the red-edge band in the index and that yield would be most accurately predicted by the CCCI, due to yield being influenced by both chlorophyll content and biomass, and the CCCI involving the red-edge band to reflect chlorophyll content and the near infrared band to detect biomass.

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## **Acknowledgements**

I would like to express her sincere gratitude to those who provided unfailing support and guidance throughout my graduate school experience. I know that without my loved ones, this thesis would not be possible.

Sincerest thanks to my major professor, Dr. Derrick Oosterhuis, for his wisdom, time, understanding, patience, enthusiasm, and encouragement. Without him, I would not have had the opportunities or the resources offered to me that I have. He is an inspiration and a role model for both career advancement and personal attitude.

Thank you to Dr. Leo Espinoza, Dr. Fred Bourland, and Dr. Curt Rom. Their guidance and knowledge helped me understand my research and gave me the drive to discover new ideas.

I would also like to acknowledge the numerous collaborators who have both given their knowledge and their funding to make this project possible: Dr. Kater Hake and the rest of Cotton Incorporated, the International Plant Nutrition Institute, the Ag Spectrum Company and the Fluid Fertilizer Foundation.

Special thanks to Dr. Tyson Raper and Dr. Cristiane Pilon, who taught me to navigate graduate school and to be a researcher. Thanks also to the numerous Crop, Soil, and Environmental Sciences graduate students who helped me with data collection and statistical analysis, as well as provided camaraderie and entertainment.

Lastly, I would like to thank my friends and family. Your support and love has been a joy in the good times, and has carried me through the bad. To my parents, Dane Coomer and Tonya Coomer, thank you for keeping me grounded while encouraging me to fly. To my grandparents and role models, Burr and Jean Swann, and Gaylon and Glenda Coomer, thank you for your always kind words and supportive prayers. Thank you all.

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# CHAPTER I

## Literature Review

### A. Importance of Potassium in Cotton

Potassium is the most abundant cation in plant cells, but is not a constituent of any single plant component (Szczierba et al., 2009; Pettigrew & Meredith, 1997). Both deficiencies and excesses can cause negative impacts on yield. Deficiency of K in cotton can cause reduced lint percentage (Pettigrew et al., 1996), plant height, leaf area (Zhao et al., 2001), dry matter production (Gerardeaux et al., 2010), lint yield (Gormus, 2002; Read et al., 2006), and termination of reproductive growth (Pettigrew, 2003). Physiologically, K is a necessary component to healthy plant water relations, stomatal opening and closing, and disease resistance (Oosterhuis et al., 2013).

### B. Potassium Requirements of Cotton

Cotton's need of K is second only to N for normal growth and development (Marschner, 1995; Oosterhuis et al., 2013). A healthy mature cotton crop contains approximately 110-250 kg K ha<sup>-1</sup>, or takes in about 2-5 kg K ha<sup>-1</sup> day<sup>-1</sup> (Bednarz et al., 1998; Halevy, 1976; Oosterhuis et al., 2013), with 54% of this K is in the vegetative organs and 46% is in the reproductive organs (Rimon, 1989). Modern cotton cultivars can require up to 4.5 kg K day<sup>-1</sup> ha<sup>-1</sup> during peak bloom (Bednarz et al., 1998; Hake et al., 1992). Critical leaf K concentration is approximately 0.95% K on a dry weight basis (Oosterhuis & Bednarz, 1997). Growth chamber and field studies show that reductions in plant growth and leaf physiological processes begin only when petiole K concentration falls below 0.88% on a dry weight basis, however, the threshold deficiency values for K concentration are questioned due to the influence of environmental factors, plant genetics, and sampling procedure (Oosterhuis & Bednarz, 1997). Tissue diagnostic recommendations,

such as those made from petiole testing, can be altered due to luxury consumption of K, or the accumulation of K beyond those needed to produce maximum yields (Oosterhuis & Bednarz, 1997). Some studies have demonstrated that luxury consumption of K can be beneficial to yields and act as a safeguard against disease and other K deficiency problems (Kafkafi, 1990; Oosterhuis, 2002).

### **C. Potassium Deficiency in Cotton**

Potassium deficiency symptoms were first observed in cotton in the US in the 1960's in the San Joaquin Valley (Gulick et al., 1989). Cotton appears to be more sensitive to K deficiencies than other crops, possibly due to poor exploitation of the soil surface layer and a less dense root system (Cope Jr., 1981; Yang et al., 2011). Potassium deficiency can affect lint yield by 10% to 50% (Brouder & Cassman, 1990; Gormus, 2002). Potassium deficiency can also affect plant growth and metabolic processes such as leaf area expansion (Zhao et al., 2001; Oosterhuis et al., 2013), dry matter production (Gerardeaux et al., 2010; Oosterhuis et al., 2013; Rosolem et al., 2003), net carbon dioxide fixation (Yang et al., 2011; Pettigrew & Meredith, 1997), fiber quality (Pettigrew & Meredith, 1997; Yang et al., 2011), boll and seed mass (Pettigrew et al., 1996), internode length (Gerardeaux et al., 2010), and N use efficiency (Pettigrew & Meredith, 1997). Excessive K, while rare, can be detrimental to growth due to increased chance of boll rot (Bennett et al., 1965; Oosterhuis et al., 2013), increased plant height (Bennett et al., 1965; Oosterhuis et al., 2013; Pettigrew & Meredith, 1997), and delayed maturity (Bennett et al., 1965; Clement-Bailey & Gwathmey, 2007; Oosterhuis et al., 2013).

Deficiency symptoms of K in cotton can be classified into two groups: classic, traditional symptoms that have been seen since the 1960's and newer symptoms that have been observed in more recent years. Due to K mobility in the plant, traditional symptoms occur at the bottom of

the plant in mature leaves then advance up the plant during late season (Dong et al., 2004).

Symptoms begin as yellow-white mottles in interveinal areas and leaf margins, then whole leaves become light yellow-green with yellow specks between veins (Hodges and Constable, 2009).

Those yellow specks become brown splotches at the leaf edges, margins, and between veins, and then leaf curl begins. Finally, the whole of the leaf becomes a reddish brown, rust color, from which the term “cotton rust” was coined, and the leaves prematurely shed (Dong et al., 2004).

Recent K deficiencies are marked by leaf discoloration and necrosis occurring in the upper canopy leaves and spreading to the bottom of the plant. These deficiencies occur during flowering and boll development (Dong et al., 2004). Three major contributing factors to the shift in K deficiency symptoms are (1) the development of higher yielding, earlier maturing cultivars which require more K over a shorter period of time than traditional, lower yielding cultivars (Oosterhuis, 1976), (2) an inefficiency of cotton roots to utilize K in the surface due to earlier-maturing cultivars failing to develop as expansive of a root system compared to later-maturing cultivars (Kerby et al., 1985), and (3) the decrease in root growth after mid-season when the boll load sink increases (Cappy, 1979). The order of plant components in which K deficiency occurs has been debated. Oosterhuis & Bednarz (1997) reported deficiency was first detected in roots, followed by stems, petioles and leaves, then fruit. However, Rosolem and Mikkelsen (1991), found the order to be stems, then roots, bolls, and petioles and leaves.

Potassium deficiencies can affect physiological processes of cotton, and can exacerbate other stresses the plant may be undergoing. Plants low in K have greater sensitivity to drought conditions, greater accumulations of sugars and ATP, and a lack of K has a strong influence on membrane activity (Hodges and Constable, 2009).

#### **D. Roles of Potassium in the Plant**

Physiologically, K is an essential macronutrient for plant growth and development. While K is not a component of any singular plant part, K affects many fundamental physiological processes such as cell pH stabilization (Marschner, 1995; Oosterhuis et al., 2013), regulating plant metabolism by acting as a negative charge neutralizer (Wang & Chen, 2012), maintaining cell turgor by acting as an osmoticum (Maathuis & Sanders, 1996; Dong et al., 2004; Szczerba et al., 2009), activating enzymes and regulating the opening and closing of stomata (Dong et al., 2004).

In a controlled environment experiment studying the effect of K deficiency during floral bud development, K-starved cotton plants had 23% of the net photosynthetic rate of K-sufficient plants; however, K-deficient leaves had a 2.3 fold higher net photosynthetic rate than the K-sufficient leaves (Zhao et al., 2001). This was probably due to a reduction in the photosynthesis system, rather than its activity (Zhao et al., 2001). The decreased net photosynthetic rate was due to lower chlorophyll content and poor chloroplast ultrastructure (Zhao et al., 2001), rather than limited stomata conductance. The total chlorophyll content of these plants was only 12% of the control, with no difference in the chlorophyll a:b ratio between the K-deficient and K-sufficient treatments (Zhao et al., 2001).

When K binds to a specific site in a protein, inactive enzymes undergo conformational change, resulting in enzyme activation of more than 60 enzymes involved in a variety of plant metabolic and physiological functions (Suelter, 1970; Marschner, 1995; Oosterhuis et al., 2013). One of these enzymes is pyruvate kinase, which plays a pivotal role in plant metabolism due to its regulation of conversion of phosphoenolpyruvate to pyruvate (Kayne, 1973; Oosterhuis et al., 2013).

Functions of K can affect functions of water in plants. Potassium in the vacuole regulates

cell osmoregulation processes (Beringer et al., 1986), as well as affecting the water potential of the cell (Hsiao & Lauchli, 1986). Turgor pressure is maintained with sufficient K levels, so K is fundamental to plant growth due to osmoregulation being crucial to cell and leaf expansion and response to drought (Maathuis & Sanders, 1996). Stomatal conductance is also decreased with less than optimum K levels (Longstreth & Nobel, 1980; Bednarz et al., 1998).

#### **E. Leaf Water Potential**

As K is a cation found readily in plants, it can directly affect both pressure potential and osmotic potential, as well as overall water potential of cells in leaves. In general, as available K supply increases, water potential decreases and osmotic potential decreases while pressure potential increases. One study using multiple cotton cultivars and K rates showed that the highest K rate of 25 g K m<sup>-2</sup> produced the lowest water potential value of -1.80 MPa (Pervez et al., 2004). Water potential in this study played a role in the number of fruits per meter, with a positive correlation coefficient of 0.93 between water potential and number of intact fruit per m<sup>2</sup>. This study also used two different K fertilizer sources, and osmotic potential decreased both due to varying K levels and K sources. On the contrary, pressure potential increased with increasing K rates. The highest K rate maintained a 35.7% higher pressure potential than the unfertilized plants (Pervez et al., 2004). Although many studies promote the idea that K rates influence water potentials in cotton leaf cells, one study (Pettigrew, 1999) showed that osmotic potential and leaf water potential were not affected by K rate, but turgor, or pressure, potential was 17% higher in leaves that received no K fertilization. This is difficult to explain, but may be related to decreased transpiration associated with K deficiency (Pettigrew, 1999).

#### **F. Stomatal Regulation**

It is understood that stomatal function is dependent upon K ions for guard cell opening

and closing. When K becomes deficient, guard cells lose K ions which in turn, decreases the pressure potential and turgidity of the cells. This decreases stomatal conductance and therefore decreases CO<sub>2</sub> uptake and photosynthesis (Wang et al., 2012). This has been shown in many experimental studies. An experiment that used four K rates, two K sources, and four cultivars showed average stomatal conductance increased with K supply regardless of cultivar or K source. The highest K fertilization rate of 25 g K m<sup>-2</sup> showed a 64.3% increase in stomatal conductance over the control 0 g K m<sup>-2</sup> rate (Pervez et al., 2004). Transpiration in this study also increased with increasing K fertilization, with maximum transpiration of 5.21 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> at the highest rate and minimum transpiration of 3.79 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> at the untreated control rate (Pervez et al., 2004). A two year experiment with four K treatments applied to one cultivar showed that while stomatal conductance declined with decreases in K fertilization ( $r^2=0.8$ ), the slope of the decline of stomatal conductance (slope=0.015) was not as steep as the decline in photosynthetic activity (0.45) (Lokhande and Reddy, 2015). This indicates that the decline in photosynthesis was not only affected by stomatal activity, but possibly a decrease in leaf chlorophyll as well (Lokhande and Reddy, 2015). A hydroponic experiment that involved two K rates and two cultivars showed an 82.8% decrease in stomatal conductance in the low K rate of 0.03 mM K from the K sufficient rate of 2.5 mM K (Wang et al., 2012). However, in a different hydroponic study, Longstreth and Noble (1980) showed little change in stomatal conductance with increasing concentrations of K.

Potassium fertilization has also been shown to aid in cotton transpiration rates in waterlogged soil conditions. When soil was waterlogged, a foliar application of K increased transpiration by 92.4% over a non-treated waterlogged condition (Ashraf et al., 2011). In the same study, a soil application of K improved transpiration by 180% and a combined foliar and

soil K application improved transpiration by 173% in waterlogged soils over non-treated waterlogged soils. However, it should be noted that normally irrigated treatment combined with the same K applications methods and rates had much higher transpiration rates than did the waterlogged soils (Ashraf et al., 2011).

#### **G. Drought Stress**

Ample K in cotton not only improves water potential and stomatal function in cotton, but can also improve water use efficiency (WUE) which helps to maintain plant function during drought stress. In a study focusing on four K doses applied to multiple cotton cultivars, water use efficiency increased as K fertilization increased (Pervez et al., 2004). The highest average WUE across all cultivars was 4.35 found at the highest K rate of 25 g K m<sup>-2</sup> and the lowest average WUE was 3.42  $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$  at the 0 K g Km<sup>-2</sup> rate (Pervez et al., 2004). This study also observed the opposite trend in canopy temperature, with the coolest canopy temperatures at the highest K rates (Pervez et al., 2004). Water use efficiency was also shown to increase with cotton cultivars in a study by Dhore et al. (2012). An application of K fertilizer increased WUE from 0.99 kg ha<sup>-1</sup> mm<sup>-1</sup> with no fertilizer to 1.26 kg ha<sup>-1</sup> mm<sup>-1</sup>. However, Tsonev et al. (2011) used two cultivars, three K rates, and three water regimes to further study the relationship between K and water stress. They found no increase in stomatal conductance by fertilizing with K in water-stressed conditions, indicating that not all cultivars or K levels can positively impact all aspects of water relations in cotton.

Yield can also be an indicator of increased tolerance by K fertilization to drought stress. Ahmad et al. (2013) used foliar K on multiple water regimes and cultivars to determine the effect of K on drought stress by observing seed cotton yield and lint quality. In both years of the study, foliar applied K increased yields for each water regime, with the maximum yield of 5.66 Mg ha<sup>-1</sup>



in the well-watered with foliar K treatment. Micronaire, an indirect measure of fiber coarseness, was increased by 0.32 with the addition of foliar K (Ahmad et al., 2013). In the water-stressed regimes, a foliar application of K reduced the intensity and effects of drought stress and improved yield. Even though each cultivar studied showed different results than other cultivars, the addition of foliar K improved lint yield and fiber quality in the less watered treatments to make them statistically equal to more well-watered regimes (Ahmad et al., 2013).

#### **H. Potassium Uptake**

The concentration of K in crop leaves is well documented, however, little is known concerning leaf K over time, especially the reproductive development period (Kafkafi & Xu, 1996). Proposed K uptake for cotton ranges from 7-22.1 kg K 100 kg lint produced<sup>-1</sup> with a suggested optimum level of 13 kg K 100 kg lint produced<sup>-1</sup> (Bennett et al., 1965; Kerby & Adams, 1985; Mullins & Burmester, 1990; Olsen & Bledsoe, 1942). Potassium is taken up through cotton roots as ion K<sup>+</sup> by diffusion and mass flow (Pimstein et al., 2011; Oosterhuis et al., 2013). Diffusion accounts for the majority of K uptake, as mass flow only accounts for 1-3% of K uptake (Marschner, 1995; Rosolem et al., 2003; Oosterhuis et al., 2013). Two-thirds of K uptake in cotton occurs in a six-week period beginning in early bloom, and maximum cumulative K uptake occurs around 112 days after planting. Potassium uptake then decreases for the rest of the growing season, due to adequate quantities in the plant and bolls, and possibly movement of K back into the soil from the plant (Gwathmey et al., 2009; Halevy, 1976). This time period is also when the plant K demand rises exponentially due to the developing boll load (Brouder & Cassman, 1990). During the growing period, K uptake by the roots satisfies cotton K demand, however, the fruiting K requirement is too high for roots to supply. The fruit K demands are met by translocation of K from leaves and shoots to bolls (Kafkafi & Xu, 1996).

The efficiency of plant uptake of K can be affected by many factors, the major of which is root surface area and root length density (Brouder & Cassman, 1990; Dong et al., 2004; Rosolem et al., 2003; Yang et al., 2011). Cotton cultivar differences in root surface area at the 0.1-0.3 m depth were positively correlated with differences in K uptake (Yang et al., 2011). Any environmental factor that restricts root growth, such as disease, insect damage, poor drainage, and compaction, reduces K uptake and may increase chances of K deficiency symptoms in the plant (Dong et al., 2004). Another set of important factors in measuring and optimizing K uptake are Michaelis-Menten uptake parameters. Two of these important parameters are  $V_{MAX}$ , defined as the maximum influx of a nutrient, and  $K_M$ , the Michaelis constant, which is the concentration of the nutrient at half of  $V_{MAX}$  (Claassen and Barber, 1974).

## **I. Potassium in Soils**

On average, soils contain 2% K, however in older or leached soils, soil K can be much less. Soil K exists in four pools: 0.1-0.2% in soil solution, 1-2% in exchangeable K, 1-10% in non-exchangeable K, and 90-98% in structural K (Ogaard et al., 2001; Rengel & Damon, 2008; Samal et al., 2010). Plant roots take up K from soil solution, which is in dynamic equilibrium with the exchangeable pool (Rengel & Damon, 2008; Samal et al., 2010). K released from the exchangeable K pool replenishes depleted soil solution K (Samal et al., 2010). Non-exchangeable K is positioned between layers of 2:1 and 2:1:1 clay minerals (Rengel & Damon, 2008). Some plant species can utilize K from the non-exchangeable pool. Sugar beet (*Beta vulgaris* L.) roots secrete exudates that can release K resulting in 7-20% higher K influx than wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) roots (Samal et al., 2010). Some potato (*Solanum tuberosum* L.) cultivars can chemically mobilize non-exchangeable K for plant use (Rengel & Damon, 2008). The main source of non-exchangeable K for maize (*Zea mays* L.)

is interlayer K in 2:1 phyllosilicate clay minerals released by cation exchange of K by  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$ , and  $\text{Na}^{+}$  accumulated in the rhizosphere (Samal et al., 2010). Smaller clay particles should release more K to soil solution due to higher surface areas (Mengel et al., 1998). Grasses may exploit interlayer K more efficiently than dicots (Mengel et al., 1998). Potassium primarily moves through diffusion in the soil (Gormus, 2002). Movement of K through soil is affected by amounts of K applied to soil, soil texture, soil cation exchange capacity, water amount, and water quality (Mallarino & Ul-Haq, 1997). The major limiting chemical component of plant growth is the lack of nutrient availability in the rooting zone (Kapur & Sekhon, 1985). Potassium status in soils is determined by removal of K in harvested products, transformations of available K to less available forms, and leaching and runoff losses (Mallarino & Ul-Haq, 1997).

#### **J. Mechanisms of Potassium Uptake**

Uptake of K from the external environment follows a biphasic pattern. Biphasic patterns are the sum of two uptake mechanisms at the plasma membrane, and each mechanism is distinguishable by saturability, and flux capacity (Szczërba et al., 2009). The two mechanisms are the high-affinity transport system (HATS) and the low-affinity transport system (LATS). The HATS is the mechanism that catalyzes the active uptake of K when K is in low concentrations, while the LATS functions at high external concentrations of K. The HATS controls the active influx of K, which is paired with the passive influx of H at a 1:1 ratio (Szczërba et al., 2009). The  $K_M$  value for HATS ranges from 10-40  $\mu\text{M}$ , and the  $V_{MAX}$  between 1.8 and 150  $\mu\text{mol g}^{-1} \text{h}^{-1}$ , depending on the plant system investigated (Maathuis & Sanders, 1996; Szczërba et al., 2009). In contrast, the channel-activated LATS causes passive influx of K with active uptake via H/K symport with consistently high  $K_M$  and  $V_{MAX}$  values at K saturation (Szczërba et al., 2009). In both mechanisms,  $\text{Na}^{+}$  suppresses K influx (Szczërba et al., 2009).

## **K. Partitioning of Potassium**

According to Mullins & Burmester (1990), mature cotton took up an average of 99-108 kg K ha<sup>-1</sup>, with 24.8% of K in the shoots, 20% of K in the leaves, 36.5% of K in the capsule walls, and 18.4% of K in the seed. In another study, Leffler (1986) found that of the K accumulated by the boll, 60% is in the capsule wall, 27% is in the seed, and 10% is in the fiber at maturity. Plant dry matter can have as much as 10% K by weight (Szczzerba et al., 2009), but the optimum amount for cotton is 2-5% (Marschner, 1995; Oosterhuis et al., 2013).

Potassium is essential for transport of carbohydrates to developing bolls (Clement-Bailey & Gwathmey, 2007). When K is limited, photosynthetic assimilate transport via the phloem is restricted (Gerardeaux et al., 2010), which, paired with limited photoassimilate production from K deficiency, leads to smaller assimilate supply to heterotrophic organs such as growing flower buds, resulting in lower yields (Clement-Bailey & Gwathmey, 2007).

Potassium in cells is stored in the vacuole and also in the cytosol at concentrations between 80 to 150 mM (Gerardeaux et al., 2010; Oosterhuis, 2002). When K is deficient, vacuolar K activity is sacrificed to maintain cytosolic K activity (Kafkafi & Xu, 1996). The highest concentrations of K are found in young developing tissues and reproductive organs indicative of high activity in cell metabolism and growth (Römheld & Kirkby, 2010).

Potassium uptake is slow during the seedling stage, increases rapidly at flowering, and slows after the maximum is reached at maturity (Oosterhuis, 2002).

Whole plant K accumulation generally follows a curve that has a maximum uptake around 112 days after planting, however, K moves throughout the plant and K concentrations in individual plant parts shift throughout the growing season (Gerardeaux et al., 2010). The K uptake curve somewhat mirrors that of dry matter production, however dry matter production

continues after K uptake has reached a maximum (Oosterhuis, 2002). Cotton bolls can accumulate K to concentrations above 40 mg/g of the dry weight (Kafkafi & Xu, 1996).

Cotton's K needs are highest during boll set because bolls are a major K sink. During the development of a boll, K concentration in plant tissue increases from 10 g kg<sup>-1</sup> to 55 g kg<sup>-1</sup> at maturity. Fiber K declines due to redistribution of K within the boll to seed and capsule wall during boll development, while seed K remains nearly constant (Oosterhuis, 2002).

#### **L. Genotypic Differences**

As K is highly mobile in plants, genotypic differences in K utilization have been associated with differences in capacity to translocate K between cells and throughout the whole plant (Rengel & Damon, 2008). Bt-transgenic cotton cultivars seem to be more sensitive to modern K deficiency than conventional cultivars, resulting in an increased interest in K fertilizers with the increased use of transgenic cotton (Dong et al., 2010). Some cultivars may be designated as K-uptake efficient, indicating those genotypes have specific physiological mechanisms to gain access to sufficient quantities of K (Rengel & Damon, 2008). K-uptake efficient genotypes may have a larger surface area of contact between roots and soil and have a greater uptake capacity at the root surface to maintain the diffusive gradient between soil and roots (Rengel & Damon, 2008). Early- and late-maturing cultivars differ in their ability to efficiently take up K from soil (Keino et al., 1995). Halevy (1976) found that early-maturing cultivars suffer from K deficiency more severely than late-maturing cultivars due to greater uptake of K during early growth and larger uptake and partitioning of K from leaves to reproductive parts. In this study, the bolls per plant and number of flowers were greater in the early-maturing cultivar (Halevy 1976).

Potassium-deficiency sensitive and K-deficiency tolerant cultivars produce similar yields

when grown under sufficient K conditions, however, when available K in soil is low, K-deficiency tolerant cultivars will produce higher yields (Brouder and Cassman, 1990). This tolerance has been associated with greater K accumulation and higher K uptake from low K soils (Brouder and Cassman, 1990). Sensitive cultivars also have less root length development and smaller root diameters than tolerant cultivars (Brouder and Cassman, 1990).

The more recent upper canopy K deficiency symptoms occur during flowering and boll development and on faster fruiting, higher yielding, early maturing, determinant cultivars more than indeterminate cultivars that mature later with lighter boll loads (Dong et al., 2004). This may be related to the sink strength of the developing boll load intercepting the K before it can reach the upper canopy younger leaves (Oosterhuis et al., 2013). These cultivars also tend to partition a larger proportion of photosynthates and nutrients to fruit load rather than new vegetative growth (Clement-Bailey and Gwathmey, 2007).

#### **M. Uses of Remote Sensing in Agronomic Row Crops**

Sensing deficiencies in the soil is usually carried out by soil and plant analysis, which can be time consuming and expensive (Ponzoni & Goncalves, 1999). It is believed that early detection of soil and plant nutrient deficiency problems can be achieved by using remote sensors that utilize the electromagnetic spectrum. Reflected and emitted energy wavelengths between 400 to 900 nm are measured by remote sensing techniques (Thomas et al., 1967). The reflecting capacity of plant canopies changes with plant species, and within a single plant species. Reflectance changes occur due to plant characteristics such as foliage density, plant height, vigor, growth habit, and maturity. Environmental effects such as salinity, moisture availability, and nutrient availability affect the radiation properties of plants by modifying plant characteristics (Thomas et al., 1967). Remotely sensed reflected energy offers a possible means

for determining crop maturity, vigor, disease, yield, moisture stress, and nutrient status of plants (Thomas et al., 1967).

To normalize spectral reflectance values, certain wavelengths are mathematically manipulated to formulate vegetation indices. Two common indices for remotely sensed reflectance values are the Normalized Difference Vegetation Index (NDVI) and the Canopy Chlorophyll Content Index (CCCI). Those wavelengths are indicated by arrows in the typical reflectance pattern for green vegetation shown in Figure 1.1.

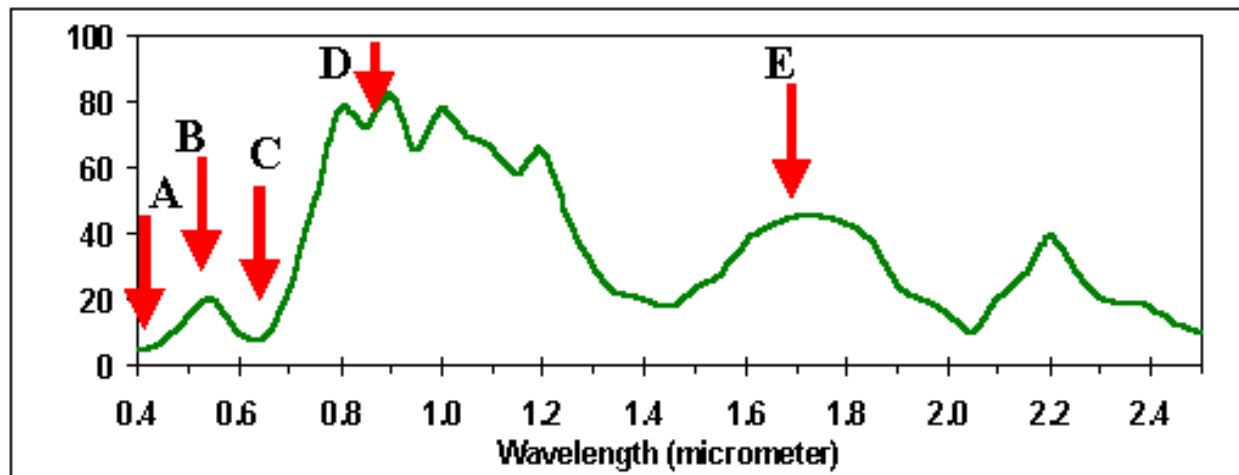


Figure 1.1. Reflectance Spectrum for general green vegetation. Image source: Center for Remote Imaging, Sensing, and Processing, 2001.

The small peak at 0.55 micrometers is the green band indicated by arrow B, the dip at 0.67 micrometer is the red band indicated by the arrow C, and the 0.76 micrometer band begins the infrared region denoted by arrow D. The sharp increasing slope between the red and infrared is called the “red-edge”, defined as the inflection point that occurs in the rapid transition between red and infrared wavelengths (Masoni et al., 1996).

#### N. Detecting Growth Stressors

Mineral deficiencies cause visible aberrations in leaf pigmentation, size, and shape, along with leaf photosynthetic rate, which is linked to the amount of absorbed radiation (Masoni et al.,

1996). Leaf external and internal reflectance, as well as leaf pigment content, represented by chlorophyll, affects absorbance of light by leaves (Masoni et al., 1996). It is accepted that nutrient deficiencies reduce leaf chlorophyll concentration, which increases leaf reflectance and transmittance, decreases leaf absorbance, and shortens the red-edge position (Masoni et al., 1996).

Gausman et al. (1969), suggested that leaf structure affected only the 750 to 1350 nm spectral range. The 700 to 1000 nm wavelengths are the near-infrared region of the electromagnetic spectrum. Thomas et al. (1966) found that as plants matured, reflectance in this region increased due to increasing cell wall-intracellular space interfaces. As a leaf ages, cell volume and therefore intracellular space increases.

The major factor affecting reflectance or absorbance in the spectral range above approximately 1350 nm is water (Gausman et al., 1969). Water especially affects the 1450 nm, 1650 nm, and 2200 nm wavelengths (Thomas et al., 1967). Thomas et al. (1966) observed an inverse relationship between leaf moisture content and leaf reflectance. Decreases in turgidity, which indicated a decrease in leaf moisture, resulted in an increase in leaf blade reflectance (Thomas et al., 1967).

Soil salinity increases cotton leaf thickness and sponginess due to the increased spongy mesophyll tissue, and salt-affected leaves are more pubescent and succulent. These morphological changes affect the absorption, reflectance, and transmittance of energy (Thomas et al., 1967). Soil salinity increased the percentage reflectance by single cotton leaves and decreased the percentage transmittance over the spectral range 500 to 2500 nm (Thomas et al., 1967). Reflectance percentages of salt-affected cotton at 550 nm, which corresponds to the pigments within chloroplasts, were lower in early season and higher in late season, than non-salt-



affected cotton, which suggests the chlorophyll content of the salt-affected leaves was higher in early season and lower in late season (Thomas et al., 1967). Salinity decreases leaf surface area overall, and therefore increases the amount of exposed soil. This explains how individual saline leaves had a higher reflectance percentage, but on a field basis, non-saline plants had a higher reflectance percentage (Thomas et al., 1967). The amount of exposed soil explains how late in the season salt-affected leaves had an increase in visible reflectance as the plant matured while non-salt-affected leaves' visible reflectance stayed constant (Thomas et al., 1967).

Leaf age can affect the spectral reflectance of leaves. In cotton leaves, leaf maturation significantly affected the 500-750 nm range as well as the 1,650-2,200 nm ranges (Gausman et al., 1971). As cotton leaves mature, especially lower leaves on nodes two through eight, intercellular spaces develop in the mesophyll that increase light reflectance and reduce transmittance. Young leaves have fewer air spaces and less reflectance than mature leaves (Gausman et al., 1971).

#### **O. Sensing Potassium Deficiency**

While the spectral reflectance curve for nitrogen (N) is well documented (Samborski et al., 2009), nutritional monitoring of other elements is not so well defined (Pimstein et al., 2011). In a study with K deficiency in grapes (*Vitis vinifera* L. cv Pinot Noir clone UC2A), researchers using the NDVI index found that taking the derivatives of the reflectance values at each wavelength, where the value of the derivative increased with increasing change in the spectra, showed some variation in K deficiency (Smart et al., 2007). When canopy reflectance was compared to wheat leaf K concentration, no interaction was found between the two. However, the K correlation followed the same pattern as the N correlation, suggesting a cross-correlation between the two elements at the leaf level (Pimstein et al., 2011). This suggests the possible need

for a biophysical parameter (N/K) in the index analysis (Pimstein et al., 2011). On a canopy scale, the relationship between K concentration and wavelength was stronger at the 1450 nm wavelength than any other wavelength (Pimstein et al., 2011). It has also been observed that more accurate predictions come from predicting total K content rather than K concentration in plants (Pimstein et al., 2011). In a study describing remote sensing of nutrient deficiencies in *Eucalyptus saligna*, results indicated that K deficiency was spectrally detected in both old and young leaves, where N and P were not detectable (Ponzoni & Goncalves, 1999).

Cotton yield response to K fertilization was significantly correlated to an increase in light interception at all layers of the canopy compared to no K fertilizer treatments (Gwathmey & Howard, 1998). Foliar K fertilization only affected canopy light interception when no soil K was applied in one of a two-year study (Gwathmey & Howard, 1998).

More information is needed on the effect of K fertilization and K deficiency on spectral reflectance curves in cotton. With more research concerning how K affects N indices, application of both nutrients will be more environmentally safe and economical.

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## CHAPTER II

### Uptake of Potassium in Cotton Plants Grown in a Hydroponic Solution

#### Abstract

Potassium uptake for cotton ranges from 7-22 kg K 100 kg lint produced<sup>-1</sup>, 97-99% of which is taken up through cotton roots by diffusion, and the rest is taken up by mass flow. Two-thirds of K uptake in cotton occurs in a six-week period beginning in early flowering, and maximum cumulative K uptake occurs around 112 days after planting. Root uptake of K is sufficient during the main vegetative period in the cotton plant's development, but the fruiting requirements of cotton are too high for the roots to take up. Root surface area and root length determines the efficiency of plant K uptake. Environmental factors can hinder a plant's K uptake. Another set of important factors in measuring and optimizing K uptake are Michaelis-Menten uptake parameters. Two of these important parameters are  $V_{MAX}$ , defined as the maximum influx of a nutrient, and  $K_M$ , the Michaelis constant, which is the concentration of the nutrient at half of  $V_{MAX}$ . This study was conducted to measure the  $V_{MAX}$  and  $K_M$  of one cultivar of cotton's K uptake at first flower (FF) and the root lengths of those plants. Results were inconclusive, as there was not a significant amount of K taken up in the time period studied.  $V_{MAX}$ ,  $K_M$  and root length were still calculated. Roots were slightly longer,  $V_{MAX}$  was lower, but  $K_M$  was higher in the high K environment. This study could be reformulated in the future for more conclusive results.

#### Introduction

Cotton uptake of K ranges from 7-22.1 kg K 100 kg lint produced<sup>-1</sup> ; optimal uptake is suggested to be 13 kg K 100 kg lint produced<sup>-1</sup> (Bennett et al., 1965; Kerby & Adams, 1985; Mullins & Burmester, 1990; Olsen & Bledsoe, 1942). Ninety-seven to ninety-nine percent of K



is taken up through cotton roots as ion  $K^+$  by diffusion and 1-3% by mass flow (Pimstein et al., 2011; Oosterhuis et al., 2013). Maximum cumulative K uptake occurs around 112 days after planting. Potassium uptake then decreases for the rest of the growing season, due to adequate quantities in the plant and bolls, and possibly movement of K back into the soil from the plant (Gwathmey et al., 2009; Halevy, 1976). There are two proposed methods of K uptake: high affinity uptake and low affinity uptake: high-affinity transport system (HATS) and low-affinity transport system (LATS). The HATS is the mechanism that catalyzes the active uptake of K when K is in low concentrations, while the LATS functions at high external concentrations of K (Claassen and Barber, 1974). Important factors in measuring and optimizing both high- and low-affinity K uptake are Michaelis-Menten uptake parameters. Two of these important parameters are  $V_{MAX}$ , defined as the maximum influx of a nutrient, and  $K_M$ , the Michaelis constant, which is the concentration of the nutrient at half of  $V_{MAX}$  (Claassen and Barber, 1974). An additional important Michaelis-Menten parameter is the  $C_{min}$ , which is the minimum concentration that the plant can use.

The main objective of this study was to determine if K concentration affected  $V_{MAX}$  and  $K_M$  of one cotton cultivar. It was hypothesized that the high K environment would lead to more K uptake by cotton roots, which would lead to an increase in  $V_{MAX}$  and  $K_M$ .

## **Materials and Methods**

This study was conducted in 2015 in the growth chambers at the Altheimer Laboratory at the University of Arkansas Division of Agriculture, Agriculture Research and Extension Center in Fayetteville. This study was set up as a randomized complete block design with six replications. The study was conducted twice to reduce the variation between the samples. Forty DeltaPine 0912 B2RF cotton (*Gossypium hirsutum* L.) seeds were germinated in vermiculite and

grown until the first true leaf emerged. Plants were culled to 36 plants, and three plants were affixed to each lid of twelve 3.74 L buckets, such that the roots of each plant were able to reach inside the bucket. Each bucket was filled with  $\frac{1}{4}$  strength Hoagland's solution so the plant roots could absorb nutrients from the solution. Buckets were placed inside a growth chamber kept at a 32 C/24 C day/night temperature with 60% humidity on 14 hour day/10 hour night photoperiod. Each bucket had a small tube threaded through a hole in the top of the bucket so that one tube end was submerged in the growth solution and the other end was attached to an aquarium air pump outside the bucket to keep the solution aerated (Figure 2.1).



Figure 2.1. Hydroponic experiment bucket set-up at time of sampling

After five days, the solution was replaced with  $\frac{1}{2}$  strength Hoagland's solution. The  $\frac{1}{2}$  strength Hoagland's solution was replaced every five days until plants reached first flower (FF). Solution was then replaced with a  $\frac{1}{2}$  strength modified Hoagland's solution without any K, to starve the plants of K before testing began. After 48 hours without K, six buckets were filled with  $\frac{1}{2}$  strength Hoagland's solution, representing a high K environment ( $0.3 \text{ M KNO}_3$ ). The

other six buckets were filled with a modified  $\frac{1}{2}$  strength solution containing  $\frac{1}{20}$  strength K ( $0.03 \text{ M KNO}_3$ ), representing a low K environment. Ten milliliter (mL) samples were taken from each bucket every hour for 12 hours, then every two hours for the next 12 hours, and then every 4 hours for the last 12 hours to observe the change in K concentration in each bucket. 10 mL of water replaced the removed solution at each sampling time. The change in K concentration was used to measure the amount of K taken up by plant roots over the 36-hour testing period. Each sample was analyzed for K concentration using inductively coupled plasma mass spectrometry (Soil and Plant Testing Laboratory, University of Arkansas, Fayetteville). Root length was found by scanning roots into the SmartRoot program (Lobet et al., 2011). To find  $V_{\text{MAX}}$  of the high and low K environments, linear equations were calculated from the plots of the changes in K concentration against time.  $V_{\text{MAX}}$  was found by taking the inverses of the y-intercepts of the lines. Multiplying the  $V_{\text{MAX}}$ 's by the slopes of the lines found  $K_M$ .

## **Results and Discussion**

The results of this study were inconclusive. There was not enough change in the K concentrations in either high or low environment to definitively conclude the differences in uptake patterns in high or low environments. Potassium was both taken up and then exuded out of the plant back into the solution multiple times over the course of the testing period. Using a different number of plants per bucket, a different starting K concentration in both low and high environments, or shortening or extending the testing period could make the study more useful for finding uptake patterns. The average root length was numerically higher in the high-K environment than the low-K environment (Table 3.1). The  $V_{\text{MAX}}$  was numerically higher in the low-K environment, but the  $K_M$  was numerically lower in the low-K environment (Table 3.1). The results from this study were not comparable to results found in literature. The  $V_{\text{MAX}}$  and  $K_M$

values in this experiment were much higher than in the literature reviewed (Szxzerba et al., 2009). This could be due to differences in initial nutrient concentrations or number of plants per growth solution container.

## Conclusions

This uptake study was inconclusive, and the results could be improved by changing the methodology of the study. The root length and  $V_{MAX}$  were higher in the high K environment, but the  $K_M$  was higher in the low K environment.

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## Appendix

Table 2.1. Differences in root length,  $V_{MAX}$ , and  $K_M$  by potassium environment for cotton plants in a hydroponic pot experiment.

<b>K Environment</b>	<b>Root Length (cm)<sub>1</sub></b>	<b><math>V_{MAX}</math> (mol/min)<sub>2</sub></b>	<b><math>K_M</math> (mol)<sub>3</sub></b>
<b>High</b>	130,904	0.435	29.064
<b>Low</b>	100,418	0.721	18.882

1. Root length is measured per bucket.
2.  $V_{MAX}$  is the maximum rate of uptake of K in by cotton roots.
3.  $K_M$  is the concentration of K at half the maximum uptake rate.

## CHAPTER III

### Potassium Partitioning Over in the Cotton Plant a Growing Season

#### Abstract

Potassium is the most abundant cation in plant cells but is not a constituent of any single plant component. Understanding the uptake and distribution of K by the cotton (*Gossypium hirsutum* L.) plant during the season is essential for efficient and profitable fertility management. Whole cotton plant K accumulation patterns have been documented for traditional non-transgenic cultivars, but there are no studies looking at modern, transgenic cultivars. A field trial was conducted over two growing seasons to observe the shift in potassium partitioning throughout the cotton plant over time. Results showed that major K shifts occurred from leaves to bolls over the growing season. However, there was little shift in the petioles or stems over a growing season. Yield was affected by both K fertilization, with the highest yields coming from a moderate K rate and the lowest yields from a 0 K rate. Yields were also separated by cultivar. DP0912 significantly out-yielded the other cultivars studied.

#### Introduction

Potassium is essential for transport of carbohydrates to developing bolls (Clement-Bailey & Gwathmey, 2007). In low-K environments, yields decrease due to lessened photosynthetic assimilate supply to growing flower buds and bolls from decreased photosynthate production and transport (Gerardeaux et al., 2010; Clement-Bailey & Gwathmey, 2007). The highest concentrations of K are found in young developing tissues and reproductive organs indicative of high activity in cell metabolism and growth (Römheld & Kirkby, 2010).

Mature non-transgenic cotton has been shown to take up an average of 99-108 kg K ha<sup>-1</sup>, with 24.8% of K in the shoots, 20% of K in the leaves, 36.5% of K in the capsule walls, and

18.4% of K in the seed (Mullins and Burmeister, 1990). Cotton's K needs are highest during boll set because bolls are a major K sink. (Oosterhuis, 2002).

The first objective of this study was to observe how K fertilization rates and cultivar affected K partitioning in the plant over a growing season. The second objective of this study was to determine if K fertilization rates and cultivar affected yield. It was hypothesized that as K fertilization increased, K uptake increases and therefore, there would be greater K shift to reproductive components due to the plant having more than enough K in all other parts enabling it to send more to the reproductive components. It was also hypothesized that greater K rates would lead to higher yields across all cultivars.

## **Materials and Methods**

The potassium partitioning study was conducted on the Lon Mann Cotton Research Station of the University of Arkansas. Soils in this trial consisted of relatively uniform Calloway Series (Fine-silty, mixed, active, thermic Aquic Fraglossudalfs). Three cultivars of cotton (*Gossypium hirsutum* L.) (DeltaPine 0912 B2RF, Phytogen 499 WRF, and Stoneville 5458 B2F) were planted in May 2014 and 2015. All fertilization besides K fertilization was applied following soil test recommendations. Four K treatments of 0, 33.6, 67.2, and 100.8 kg K/ha (0, 30, 60, and 90 lb K/acre) were applied as potassium chloride (KCl) to each of the three cultivars at approximately pinhead square (PHS), resulting in twelve overall treatments. Plots were four 1 m (38 inches) rows wide and 15.24 m (50 feet) long. Plots were furrow irrigated as needed.

One meter of whole plants was sampled from four replications from each of the 12 treatments at PHS, first flower (FF), three weeks after first flower (FF3), and six weeks after first flower (FF6). SPAD meter (Konica Minolta, Tokyo, Japan) readings were also taken at these growth stages as an estimate of chlorophyll content. Whole plant samples were then broken



down into four main plant components: stems, leaves, petioles, and reproductive components. These plant components were dried at 60°C for at least one week, weighed, ground, and sent to AGVISE Laboratories (Benson, MN) in 2014 and the University of Arkansas Soil and Plant Testing Laboratory (Fayetteville, AR) in 2015, where they were analyzed for K concentration by nitric peroxide digestion, and K levels were determined using inductively coupled plasma atomic emission spectroscopy. Yield data were measured at harvest on October 23 in 2014 and October 5 in 2015, and included lint yield, boll number, boll weight, and fiber characteristics.

### *Statistical Analysis*

This experiment was analyzed as a 2 factor factorial completely randomized design with four replications with data combined over years using the year as a random variable. Statistics were analyzed using JMP Pro 11 (SAS Institute, Cary, North Carolina) with an alpha level of 0.05 as an indication of significance. Differences between treatments were determined using a student's t-test.

## **Results and Discussion**

### **A. Cultivar Differences at Each Potassium Level Throughout a Growing Season**

Before partitioning data were analyzed, outliers were determined using the multivariate method of jackknife distances. Data was analyzed as a two factor factorial with year as a random variable.

#### ***Leaves***

No matter the cultivar (not shown) or K level, percent of total plant K in leaves increased significantly ( $p < 0.05$ ) at each growth stage throughout the growing season (Table 4.1). There were no cultivar differences in percent of total plant K in leaves at any K level. At 0 kg K/ha, PHS had the highest percent of total plant K in leaves with a mean percentage of 55.15%, and

decreased throughout the growing season and ended at 15.68% at FF6. At 33.6 kg K/ha, the same trend occurred with the highest mean percent of total K in leaves at PHS with 57.25% K in leaves and decreased to 9.61% at FF6. At 67.2 kg K/ha, PHS mean percent total plant K in leaves was 52.48 and decreased to 14.66% at FF6. Finally, at 100.8 kg K/ha, PHS mean percent total plant K in leaves was 58.38% and decreased to 10.41% at FF6.

### ***Petioles***

Similar to the percent total K in leaves, there was no cultivar differences at any K level. Partitioning of K to petioles is shown in Table 4.1. The 0 kg K/ha applied treatments only had a significant ( $p<0.05$ ) difference between growth stages when the highest numerical mean percent total K in petioles at PHS was contrasted with the lowest numerical mean percent total K in petioles FF3. At 33.6 kg K/ha, the highest mean percent total K in petioles was at PHS at 14.78% and decreased throughout the growing season to 3.1% at FF6. At 67.2 kg K/ha, the PHS and FF growth stages had significantly ( $p<0.05$ ) higher mean percent total K in petioles than FF3 and FF6. There were no differences between growth stages at 100.8 kg K/ha. Data is shown in Table 4.1.

### ***Reproductive Components***

Reproductive component K partitioning showed no significant ( $p<0.05$ ) differences between cultivars at any K level, and only showed an interaction at 67.2 kg K/ha treatments (Table 4.1). Growth stage was significant ( $p<0.05$ ) at each K level. Reproductive component K partitioning significantly ( $p<0.05$ ) increased over the growing season at each K level. For the 0 kg K/ha treatment the FF6 stage had, on average, 72.78% percent of total K in reproductive components, and only 1.9% percent of total K in RC at PHS. At 33.6 kg K/ha, mean percent of total K in reproductive components began at 2.45% at PHS and increased to 59.31% at FF6. The

only significant ( $p < 0.05$ ) interaction of cultivar and K level in partitioning by K level occurred in reproductive components with 67.2 kg K/ha applied. The same general trend occurred where mean percent of total K in reproductive component increased over the growing season, but cultivar Delta Pine 0912 B2RF had the highest mean percent in reproductive components of the three cultivars at PHS and FF, but by FF6, had the lowest percent in reproductive components of the cultivars. At PHS, FF, and FF3, cultivar PHY 499 WRF partitioned the least K to reproductive components of the three cultivars, but at FF6, had the highest mean percent K of all cultivars. With 100.8 kg K/ha applied, PHS and FF were significantly ( $p < 0.05$ ) the same, and also lower than FF3 and FF6 with 4.76, 8.33, 41.78, and 66.58% total K in reproductive components, respectively (Table 4.1).

### ***Stems***

The only significant ( $p < 0.05$ ) differences when analyzing K partitioning to stems occurred between growth stages (Table 4.1). No cultivar differences or interaction between cultivar or growth stage were significant ( $p < 0.05$ ). Total percent K in stems follows a general trend at each K level, where stem K peaks at FF, and is lowest at the end of the growing season. The 0 kg K/ha treatments had a mean percent of total K of 45.98% in stems at FF and of 16.78% at FF6. At 33.6 kg K/ha, mean percent of total K was 49.12 and 18.65% at FF and FF6, respectively. Mean percent of total K in stems with 67.2 kg K/ha applied was 48.92% at FF and 20.27% at FF6. PHS, FF3, and FF6 were all significantly ( $p < 0.05$ ) the same in the 100.8 kg K/ha treatments, and all lower than FF, with 35.68, 32.30, 29.84 and 43.68%, respectively (Table 4.1).

### ***Transgenic and Non-Transgenic Cultivars***

Previously studied non-transgenic cultivars partitioned around 25% total plant K to stems while modern, transgenic cultivars studied in this experiment only partitioned between 12 and

20% (Mullins and Burmeister, 1990). The aforementioned experiment with older, conventional, non-transgenic cultivars showed that those cultivars partitioned more K to leaves than the current study (approximately 20% compared to 10-15%) and less K to reproductive units (approximately 55% compared to 62-67%) (Mullins and Burmeister, 1990).

## **B. SPAD (Chlorophyll)**

There were no significant ( $p < 0.05$ ) cultivar differences or cultivar and growth stage interactions, and no difference between K levels within each growth stage of SPAD readings (data not shown), however, there were significant ( $p < 0.05$ ) growth stage differences at the 62.7 and 100.8 kg K/ha levels. At the 0 kg K/ha level, mean SPAD readings were not significantly ( $p < 0.05$ ) different from one another using the student's t- test. However, when contrasts were used, there were significant ( $p < 0.05$ ) differences between the numerically highest SPAD reading at FF3 and each other growth stage with p levels of 0.0178 for the contrast between FF3 and FF, 0.0458 for the contrast between FF3 and FF6, and 0.011 between FF3 and PHS. When K levels were slightly higher at 33.6 kg K/ha, the same trend continued, where contrasts between growth stages revealed significant ( $p < 0.05$ ) p values between FF3 and FF of 0.0238, FF3 and FF6 of 0.0178, and FF3 and PHS of 0.014. At the 67.2 kg K/ha level, FF3 had significantly ( $p < 0.05$ ) higher mean SPAD readings than FF, PHS, and FF6. The highest K level had highest SPAD levels at FF3 and lowest at FF and PHS. Data are found in Table 4.2.

## **C. Yield Data**

Before yield data were analyzed, outliers were determined and discarded from analysis. Lint yield showed significant ( $p < 0.05$ ) differences at the 0.05 alpha level for both main effects of K level (Table 4.3) and cultivar (Table 4.4), but not for the interaction between the two. The highest yielding K level was 67.2 kg K/ha with an average yield of 1287 kg lint/ha, which was

significantly larger than the 33.6 kg K/ha and 0 kg K/ha treatments (Table 4.3). The DP 0912 B2RF cultivar significantly out-yielded the other two cultivars with a yield of 1235 kg lint/ha (Table 4.4).

#### **D. Fiber Characteristics**

Fiber characteristics were measured using high volume instrumentation (HVI) (Cotton Fiber Lab, Louisiana State University AgCenter, Baton Rouge, LA). Characteristics include fiber length, fiber strength, and micronaire, or fineness of fibers. Significant ( $p < 0.05$ ) differences were found in both K level and cultivar and in the interaction between K level and cultivar for all three characteristics.

##### ***Fiber Length***

The interaction between K level and cultivar was significant ( $p < 0.05$ ). All four K levels of cultivar PHY499 WRF were numerically longer than all four K levels of cultivar ST5458, and both were numerically longer than all four K levels of cultivar DP0912 B2RF. However, the length of K levels within each cultivar is not consistent. Cultivar PHY499 WRF with 33.6 kg K/ha had a mean length of 2.9 cm and was significantly ( $p < 0.05$ ) longer than all other treatments. Cultivar DP 0912 B2RF with 33.6 kg K/ha had the shortest mean fiber length at 2.74 cm (Table 4.5).

##### ***Fiber Strength***

Fiber strength is measured in grams-force/tex (g/tex). Both interaction and main effects were significant at the  $p > 0.0001$  level. Interaction results closely follow length results, where cultivars PHY499 WRF, ST 5458 B2F, and DP0912 B2RF are separated in that order, but K levels are not consistent. The strongest mean fibers were found in cultivar PHY499 at 0 and 33.6 kg K/ha applied with 32.56 and 38.65 g/tex, respectively. The weakest fibers were found in the

DP0912 B2RF cultivar with 67.2 kg K/ha having the lowest strength at 29.59 g/tex (Table 4.5).

### ***Micronaire***

Fiber micronaire is a measure of fiber fineness and maturity. Premium micronaire values range from 3.7-4.2, while base micronaire values range from 3.5-3.6 and 4.3-4.9. Micronaire values below 3.4 or higher than 5.0 qualify as discount, or poorest quality cotton (Cotton, Inc., Cary, NC). Micronaire measurements showed significant ( $p < 0.05$ ) differences between the interaction of K level and cultivar, and for both main effects of K level and cultivar. Overall, four treatment combinations had mean micronaire values that qualified as premium, while the other eight treatment combinations had mean micronaire values that qualified as base. No treatment was out of these ranges, or would be labeled as discount (Table 4.5). The four premium micronaire treatments were cultivar DP 0912 B2RF with 0 and 100.8 kg K/ha applied and cultivar ST 5458 B2F with 67.2 and 100.8 kg K/ha applied.

### **Conclusions**

Major K shifts occurred in the leaves and reproductive component from PHS to FF6. The proportion of total K in the leaves significantly decreased throughout the season in every treatment, however, there were no differences among any treatments at each growth stage ( $p < 0.05$ ). Regardless of treatment, the proportion of total K in reproductive components significantly increased throughout the season ( $p < 0.05$ ). Although the proportion of total K in reproductive components increased drastically over the growing season, the overall concentration of K in reproductive components decreased due to the increase in biomass from PHS to FF6 (not shown). Yield was affected by both K fertilization rate and cultivar, but not by the interaction of the two. Fiber quality was affected by the interaction of cultivar and K fertilization rate, however only two of three cultivars produced yield that produced premium

micronaire fiber.

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## Appendix

Table 4.1. Partitioning of K by leaves, petioles, reproductive components and stems measured over three cultivars of cotton treated with four K levels for four growth stages in the 2014 and 2015 growing season.

Percent of Whole Plant K in Each Plant Part per K Level				
Leaves				
K Level kg K/ha	PHS	FF	FF3	FF6
0	53.15 a <sub>1</sub>	32.51 b	25.52 c	13.57 d
33.6	53.20 a	31.52 b	23.67 c	15.61 d
67.2	51.21 a	32.99 b	23.79 c	11.74 d
100.8	48.38 a	35.11 b	24.11 c	10.41 d
Petioles				
K Level kg K/ha	PHS	FF	FF3	FF6
0	12.30 a	12.23 a	08.13 b	06.87 a
33.6	14.05 a	09.16 b	07.48 b	05.05 c
67.2	13.75 a	13.30 a	04.97 b	03.41 b
100.8	14.07 a	11.30 a	06.01 b	05.39 b
Reproductive Components				
K Level kg K/ha	PHS	FF	FF3	FF6
0	01.90 d	09.28 c	35.98 b	62.78 a
33.6	02.45 d	10.12 c	37.80 b	67.31 a
67.2	03.60 d	09.33 c	39.78 b	64.58 a
100.8	04.60 c	08.91 c	41.78 b	64.58 a
Stems				
K Level kg K/ha	PHS	FF	FF3	FF6
0	32.65 ab	45.98 a	30.37 b	16.78 c
33.6	30.30 b	49.12 a	31.05 b	12.03 c
67.2	31.44 b	44.38 a	31.46 b	20.27 c
100.8	32.31 b	44.68 a	29.10 b	19.62 c

1. Lowercase letters indicate differences within each row for each stage at p=0.05.



Table 4.2. SPAD measurements of four K fertilization rates across cultivars in the 2014 and 2015 growing seasons.

<b>K Level kg K/ha</b>	<b>SPAD units</b>			
	PHS	FF	FF3	FF6
0	50.88 a <sub>1</sub>	50.91 a	55.18 a	51.65 a
33.6	50.92 a	51.28 a	55.08 a	51.08 a
67.2	49.03 a	49.52 a	52.76 b	48.93 a
100.8	48.88 a	49.58 a	55.81 a	50.58 ab

1. Lowercase letters indicate differences within each row at p=0.05.

Table 4.3. Cotton lint yield of four K fertilization rates in the 2014 and 2015 growing seasons.

<b>K Level kg K/ha</b>	<b>Lint Yield</b>
0	1159 c <sub>1</sub>
33.6	1207 bc
67.2	1287 a
100.8	1232 ab

1. Letters indicate differences within columns at p=0.05.

Table 4.4. Cotton lint yield of three cultivars in the 2014 and 2015 growing seasons.

<b>Cultivar</b>	<b>Lint Yield</b>
PHY499	1109 b <sub>1</sub>
ST4548	1132 b
DP0912	1235 a

1. Letters indicate differences within columns at p=0.05.

Table 4.5. Cotton fiber characteristics of four K fertilization rates and three cultivars across the 2014 and 2015 growing seasons.

Fiber Characteristics			
Length (cm)			
K Level kg K/ha	PHY499	ST5458	DP0912
0	2.82 b <sup>1</sup>	2.79 bc	2.73 de
33.6	2.90 a	2.77 c	2.69 f
67.2	2.82 b	2.76 cd	2.70 ef
100.8	2.82 b	2.81 bc	2.71 ef
Strength (g/tex)			
K Level kg K/ha	PHY499	ST5458	DP0912
0	26.21 a	32.25 e	31.14 f
33.6	35.55 ab	31.33 f	30.82 f
67.2	35.30 bc	33.81 d	30.54 f
100.8	34.45 cd	33.69 d	31.34 f
Micronaire (Quality)			
K Level kg K/ha	PHY499	ST5458	DP0912
0	4.63 (B) <sup>2</sup> bcd	3.66 (B) f	4.20 (P) f
33.6	4.66 (B) abc	4.47 (B) cde	4.46 (B) de
67.2	4.84 (B) a	3.82 (P) f	4.73 (B) ab
100.8	4.43 (B) e	4.11 (P) f	4.18 (P) f

1. Letters indicate significant ( $p < 0.05$ ) differences between all treatments of each section.
2. Letters inside parenthesis denote micronaire quality where P mean premium and B means base.

## **CHAPTER IV**

### **Use of Remote Sensing in Cotton to Determine Potassium Status and Yield**

#### **Abstract**

Cotton has a less dense rooting system than most other row crops, and therefore is less efficient at extracting nutrients such as K that move through the soil by diffusion. It has been widely determined that N deficiency can be determined using spectral reflectance indices before deficiency symptoms are visible on the plant. However, these indices are also sensitive to detecting other plant growth stressors including drought stress and other nutrient deficiency. If producers use these indices to detect N deficiency, when in reality, other stressors are affecting the index readings, producers would apply unnecessary N fertilizer which is environmentally and economically costly. The goal of this study was to observe if indices used to determine N deficiency were sensitive to K deficiency in cotton. A two-year study was conducted at the Lon Mann Cotton Research Center where spectral reflectance readings were taken at first flower (FF) and three weeks after first flower (FF3). These reflectance readings were transformed into three indices, the normalized difference vegetation index (NDVI), normalized difference red edge (NDRE), and canopy chlorophyll content index (CCCI), to study the correlation between index reading and leaf K concentration and available  $K_2O$  in the soil, as well as between yield at the end of season and index reading to observe trends in index reading and yield. Results showed that leaf K concentration was most accurately determined by the NDVI early in the season, but was also significantly correlated with later season readings. Available  $K_2O$  was not detected by reflectance indices at either growth stage studied. Yield was best predicted by the CCCI later in the season. These results indicate that reflectance sensors can be a helpful tool in determining K status of cotton leaves.

## Introduction

Sensing deficiencies in the soil is usually carried out by soil and plant analysis, which can be time consuming and expensive (Ponzoni & Goncalves, 1999). It is believed that early detection of soil and plant nutrient deficiency problems can be achieved by using remote sensors that utilize the electromagnetic spectrum. Reflected and emitted energy wavelengths between 400 to 900 nm are measured by remote sensing techniques (Thomas et al., 1967). The reflecting capacity of plant canopies changes with plant species, and within a single plant species.

Reflectance changes occur due to plant characteristics such as foliage density, plant height, vigor, growth habit, and maturity. Environmental effects such as salinity, moisture availability, and nutrient availability affect the radiation properties of plants by modifying plant characteristics (Thomas et al., 1967). Remotely sensed reflected energy offers a possible means for determining crop maturity, vigor, disease, yield, moisture stress, and nutrient status of plants (Thomas et al., 1967). While the spectral reflectance curve for nitrogen (N) is well documented (Samborski et al., 2009), nutritional monitoring of other elements is not so well defined (Pimstein et al., 2011). In a study describing remote sensing of nutrient deficiencies in *Eucalyptus saligna*, results indicated that K deficiency was spectrally detected in both old and young leaves, where N and P were not detectable (Ponzoni & Goncalves, 1999). Cotton yield response to K fertilization was significantly correlated to an increase in light interception at all layers of the canopy compared to no K fertilizer treatments (Gwathmey & Howard, 1998).

It was hypothesized that NDVI would more accurately predict leaf K, available K<sub>2</sub>O, and yield than the NDRE, due to the red-edge band used in the NDRE reflecting changes in chlorophyll, which is not affected by K deficiency. It was also believed that the NDVI and the NDRE would more accurately determine the K parameters chosen than the CCCI, due to the

strong influence of the red-edge band in the index. Yield would be most accurately predicted by the CCCI, due to yield being influenced by both chlorophyll content and biomass, and the CCCI involving the red-edge band to reflect chlorophyll content and the near infrared band to detect biomass. Therefore, the objectives of this study were to determine if cultivars differed in values from currently available indices formulated for N-status detection from active sensors. It also set out to determine if these N-sensitive indices were sensitive to leaf K concentration and available K<sub>2</sub>O in the soil, and to evaluate the role these indices play in predicting yield.

## **Materials and Methods**

The early detection of K deficiency using remote sensing experiment was conducted on the Lon Mann Cotton Research Station of the University of Arkansas. Soils in this trial consisted of relatively uniform Calloway Series (Fine-silty, mixed, active, thermic Aquic Fraglossudalfs). Soil samples were taken from shoulders of beds in each plot and analyzed for nutrient concentration, pH, and organic matter. Three cultivars of cotton (*Gossypium hirsutum* L.) (DeltaPine 0912 B2RF, Phytogen 499 WRF, and Stoneville 5458 B2F) were planted in mid May 2014 and 2015. All fertilization besides K fertilization was applied following soil test recommendations. Four K treatments of 0, 33.6, 67.2, and 100.8 kg K/ha (0, 30, 60, and 90 lb/acre) were applied as potassium chloride (KCl) at approximately pinhead square (PHS) on June 25. Plots were four 1 m (38 inches) rows wide and 15.24 m (50 feet) long with cotton planted 11.5 plants per meter (3.5 plants per foot). Plots were furrow irrigated as needed.

Spectral reflectance measurements were taken at first flower (FF) and three weeks after first flower (FF3) using a Crop Circle ACS-470 sensor with a GeoSCOUT GLS-400 data logger (Holland Scientific, Inc., Lincoln, NE). Sensor was held at 0.914 m (36 inches) above canopy. Measurements were taken by holding the sensor above the canopy and walking between the first

and second row and the third and fourth row of each plot between 10:00 AM and 2:00 PM. Wavelengths measured included 650 nm (red), 720 nm (red-edge), and 840 nm (near infrared [NIR]). Three indices from these wavelengths were calculated. The NDRE was calculated by subtracting the measurements from the red-edge from the NIR and dividing that by the sum of the measurements from red-edge and the NIR (Figure 5.1). The NDVI was calculated from subtracting the measurements from the red from the NIR, and dividing that by the sum of the measurements from the red and the NIR (Figure 5.1). The CCCI calculated by dividing the NDRE by the NDVI (Figure 5.1).

$$NDVI = \frac{R_{NIR} - R_{RED}}{R_{NIR} + R_{RED}}$$

$$NDRE = \frac{R_{NIR} - R_{EDGE}}{R_{NIR} + R_{EDGE}}$$

$$CCCI = \frac{NDRE}{NDVI}$$

Figure 4.1. Equations used to form the NDVI, NDRE, and CCCI indices.

The NDRE was used to estimate chlorophyll content, where the NDVI was used to estimate canopy cover. The CCCI takes both of these estimations to make a multi-dimensional measurement of plant health. Data points were taken using a GPS attached to the data logger so that points could be assigned to their respective plots using ArcGIS 10.2.2 (ESRI, Redlands, CA).

Available K<sub>2</sub>O was calculated using soil K concentration and fertilizer rate in each plot. Leaf samples were taken from the fourth main-stem node from the top of five plants in each plot and were analyzed for K concentration (Soil and Plant Testing Laboratory, University of

Arkansas, Fayetteville, AR). Leaf K concentration and available K<sub>2</sub>O were compared to spectral index measurements to determine the accuracy of spectral reflectance values to determine K deficiency. Lint yield from the middle two rows per plot was also recorded at harvest and was compared to index measurements to observe any correlation between spectral reflectance and yield.

### *Statistical Analysis*

This experiment was a completely randomized design with replications varying between four and eight, due to the layout of the trial. Correlations between K concentration, available K<sub>2</sub>O, and yield with cultivar as an additional main effect at each growth stage were determined using linear regression analysis in JMP Pro 11 with an alpha level of 0.05. Year was added as a random variable. Before data were analyzed, outliers were determined multivariate jackknife distances and excluded.

### **Results and Discussion**

The NDVI was significantly correlated ( $p < 0.05$ ) with the interaction between cultivar and leaf K concentration at FF with an  $r^2$  value of 0.815 (Table 5.1). The NDRE was also significantly ( $p < 0.05$ ) correlated with the interaction between cultivar and leaf K concentration at FF with an  $r^2$  value of 0.617 (Table 5.1). The significant interaction indicates that to accurately determine K status using the NDVI or NDRE, a cultivar correction factor must be used. The CCCI was not significantly correlated ( $p < 0.05$ ) with leaf K concentration at FF (Table 5.1). At FF3, no interaction between cultivar, leaf K and NDVI was significant, however, the NDRE and the CCCI had significant correlations ( $p < 0.05$ ) with cultivar with  $r^2$  values of 0.335 and 0.689, respectively (Table 5.1). This indicates NDRE and CCCI differ by cultivar, regardless of leaf K status. The leaf K concentration range at FF3 was 0.4-1.2%, well below the sufficient leaf K

range of 2-4%. It is likely that leaf K was too low overall at the FF3 stage for the spectral reflectance indices to detect leaf K status. These results are different in that research conducted using the same indices and wheat showed no correlation between index values and leaf K concentration (Pimstein et al., 2011). This study could have been improved by taking K concentrations from both young and old leaves, like the study done by Ponzoni and Goncalves (1999). In their study, K deficiency was detected in both ages of leaves, while this study only studied young leaves at the top of the canopy.

There were no significant correlations ( $p < 0.05$ ) between available  $K_2O$  and index values (Table 5.2). This could be due to the long-term K fertility research history of the field used. It is suspected that available  $K_2O$  was too low for spectral reflectance indices to detect differences. However, there were significant ( $p < 0.05$ ) cultivar by NDVI relationships at FF and cultivar by NDRE and CCCI relationships at FF3, indicating that regardless of available  $K_2O$ , cultivars differed in index values (Table 5.2).

Index values at FF and FF3 were correlated with yield data to observe if it was possible to use spectral reflectance data to predict yield early- or late-season. All three indices had significant interactions between cultivar and yield at FF and FF3 (Table 5.3). At FF, the NDVI, NDRE, and CCCI had  $r^2$  values of 0.311, 0.339, and 0.201, respectively. At FF3, the NDVI, NDRE, and CCCI had  $r^2$  values of 0.338, 0.277, and 0.693, respectively (Table 5.3). The highest  $r^2$  value was observed using the CCCI at FF3. Yield was best predicted later in the season and using an index that involves both bands that reflect changes in chlorophyll and biomass. These results were similar to another study where yield was correlated to K fertilization in an experiment using cotton (Gwathmey & Howard, 1998). However, the results from this study correlated yield to index values across all K fertilization rates, while the other study only found



correlation between low K treatments and index values (Gwathmey & Howard, 1998).

## Conclusions

Overall, leaf K concentration was best described using early-season NDVI with a cultivar correction factor. Late-season K concentrations were too low for accurate detection of significant differences. The indices chosen for this experiment were unable to determine available K<sub>2</sub>O in the soil, possibly due to the long-term fertility research field history. Yield was best predicted using the CCCI with a cultivar correction factor later in the season. These results indicate that N-sensitive indices are sensitive to other crop growth parameters, and that more research needs to be conducted to further understand the role of spectral reflectance sensors in crop production.

## References

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## Appendix

Table 4.1. Cultivar and leaf K% correlated with NDVI, NDRE, and CCCI at first flower (FF) and three weeks after first flower (FF3) in the 2014 and 2015 growing seasons.

Growth Stage	Effect	NDVI		NDRE		CCCI		
FF	Cultivar	0.0343 <sup>1</sup>	r <sup>2</sup> =0.815 <sup>3</sup>	NS <sup>1</sup>		NS		
	Leaf K%	0.0274		0.395	r <sup>2</sup> =0.617	NS		
	Cult * Leaf K%	0.0014		0.0087		NS		
FF3	Cultivar	NS		0.0058	r <sup>2</sup> =0.335	0.0131	r <sup>2</sup> =0.689	
	Leaf K%	NS		NS		NS		
	Cult * Leaf K%	NS		NS		NS		

1. Numbers in these columns indicate p-values
2. NS=Not Significant at  $p<0.05$
3.  $r^2$  values represent the interaction between main effects when interaction is significant.

Table 4.2. Cultivar and available K<sub>2</sub>O correlated with NDVI, NDRE, and CCCI at first flower (FF) and three weeks after first flower (FF3) in the 2014 and 2015 growing seasons.

Growth Stage	Effect	NDVI		NDRE		CCCI	
FF	Cultivar	0.0472 <sup>1</sup>	r <sup>2</sup> =0.798	NS <sup>2</sup>		NS	
	Available K <sub>2</sub> O	NS		NS		NS	
	Cult *Avail K <sub>2</sub> O	NS		NS		NS	
FF3	Cultivar	NS		0.0058	r <sup>2</sup> =0.335	0.0131	r <sup>2</sup> =0.689
	Available K <sub>2</sub> O	NS		NS		NS	
	Cult *Avail K <sub>2</sub> O	NS		NS		NS	

1. Numbers in these columns indicate p-values
2. NS = Not Significant at  $p<0.05$

Table 4.3. Yield predicted by NDVI, NDRE, and CCCI at first flower (FF) and three weeks after first flower (FF3) in the 2014 and 2015 growing seasons.

Growth Stage	Effect	NDVI		NDRE		CCCI	
FF	Cultivar	NS <sub>1</sub>		NS		NS	
	Yield	<0.0001 <sub>2</sub>	$r^2=0.311^3$	<0.0001	$r^2=0.339$	NS	
	Cult * Yield	0.0009		0.0032		0.0019	$r^2=0.201$
FF3	Cultivar	0.0004	$r^2=0.338$	0.0003	$r^2=0.227$	0.0036	$r^2=0.693$
	Yield	0.0408		NS		NS	
	Cult * Yield	<0.0001		0.0031		0.0056	

1. Numbers in these columns indicate p-values
2. NS = Not Significant at  $p<0.05$
3.  $r^2$  values represent the interaction between main effects when interaction is significant.

## **CHAPTER V**

### **Conclusion**

In conclusion, the uptake experiment in this study was not conclusive, therefore the hypothesis was rejected. This study could be further improved by changing the methodology to better fit the objectives of the study. More replications and more cultivars studied would allow for comparison between cultivars, as opposed to just the one studied in this experiment. The partitioning study data led to a failure to reject the hypothesis. There was a large shift from leaves in early season to reproductive components in the late season. Higher rates of K fertilization led to a greater K uptake and greater shift in K partitioning to reproductive components. The spectral reflectance study also led to a failure to reject the hypothesis. The early-season NDVI was most correlated with leaf K concentrations, and late season CCCI best predicted yield.